WO 2005/003775 PCT/IB2004/002657

144

CLAIMS

1. A conjugate molecule comprising an oligo- or polysaccharide selected from the group consisting of:

 $(X)_{x}-\{B(E)C\}-(Y)_{y}$ 5 $(X)_{x}-\{(E)CD\}-(Y)_{v}$ $(X)_x$ -{AB(E)C}-(Y)_v $(X)_{x}$ -{B(E)CD}-(Y)_v $(X)_{x}$ -{(E)CDA}-(Y)_y $(X)_{x}$ -{DAB(E)C}n- $(Y)_{y}$ $(X)_x$ -{B(E)CDA}n-(Y)_v 10 $(X)_x$ -{(E)CDAB}n-(Y)_v $(X)_x$ -{AB(E)CD}n-(Y)_y $(X)_x$ -{DAB(E)CD}-(Y)_v $(X)_x$ -{B(E)CDAB(E)C} -(Y)_y wherein: 15 A is an alphaLRhap-(1,2) residue B is an alphaLRhap-(1,3) residue C is an alphaLRhap-(1,3) residue E is an alphaDGlcp-(1,4) residue D is a betaDGlcNAcp-(1,2) residue 20 x and y are independently selected among 0 and 1

X and Y are independently selected among A, B, C, D, E, AB, B(E), (E)C, CD, DA, AB(E), B(E)C, (E)CD, CDA, AB(E)C, B(E)CD, (E)CDA, CDAB, DAB(E)

and wherein n is an integer comprised between 1 and 10 covalently bound to a carrier.

- 2. A molecule according to claim 1 wherein n is comprised between 2 and 6.
- 3. A molecule according to claim 1 wherein the carrier is selected among a protein or a peptide comprising at least one T-cell epitope, or a derivative thereof.
 - 4. A molecule according to claim 3, wherein the carrier is the peptide
- 30 PADRE.

25

35

- 5. A molecule according to claim 3, wherein the carrier is the tetanus toxoid.
 - 6. A molecule according to claim 1, wherein the carrier is biotin.
- 7. A molecule according to claim 1, wherein the saccharide is directly bound to the carrier.
- 8. A molecule according to claim 1, wherein the saccharide is bound to the carrier via a spacer.

5

15

35

- 9. A molecule according to claim 1, wherein the saccharide to carrier ratio is comprised between 1:1 and 30:1.
- 10. A molecule according to claim 1, wherein the saccharide is selected among the tetrasaccharides and pentasaccharides and their oligomers.
- 11. A molecule according to claim 1, wherein the saccharide is {B(E)CD}.
 - 12. A molecule according to claim 1, wherein the saccharide is {AB(E)CD}n and n is an integer selected from 2, 3.
- 13. An immunogenic composition comprising a molecule according to anyone of claim 1 to claim 12 and a physiologically acceptable vehicle.
 - 14. The composition of claim 13, wherein said oligo- or polysaccharide is a pentasaccharide or a multimer thereof such as a decasaccharide or a pentadecasaccharide
 - 15. The composition of claim 13 or claim 14, comprising an immunogen which afford protection against another pathogen, such as for example, S. flexneri serotype 1b, 3a and 6, S. species such as S. dysenteriae and S. sonnei or pathogens responsible for diarrhoeal disease in humans.
 - 16. The composition of anyone of claims 13 to 15, which is formulated for parenteral, oral or intranasal administration.
- 17. A kit for the diagnostic of Shigella flexneri type 2a infection, wherein said kit comprises a molecule according to anyone of claim 1 to claim 12.
 - 18. A saccharide selected from the group consisting of:

 $\{B(E)CD\}$

 $\{(E)CDAB\}n$

 ${AB(E)CD}n$

25 wherein:

A is an alphaLRhap-(1,2) residue

B is an alphaLRhap-(1,3) residue

C is an alphaLRhap-(1,3) residue

E is an alphaDGlcp-(1,4) residue

D is a betaDGlcNAcp-(1,2) residue

And n is an integer comprised between 1 and 10.

19. A saccharide derivative selected from the group consisting of:

 $\{B(E)CD\}$ -OQ

{(E)CDAB}n-OQ

 ${AB(E)CD}n-OQ$

{DAB(E)C}m-OQ

 $\{B(E)CDA\}m-OQ$

{DAB(E)CD}- OQ

of:

146

wherein: A is an alphaLRhap-(1,2) residue B is an alphaLRhap-(1,3) residue C is an alphaLRhap-(1,3) residue 5 E is an [alphaDGlcp-(1,4)] residue D is a betaDGlcNAcp-(1,2) residue Q is selected among alkyl and alkenyl groups comprising 1 to 12 carbo atoms, O is an oxygen atom n is an integer comprised between 1 and 10 and 10 m is an integer comprised between 2 and 10. 20. A pharmaceutical composition comprising a molecule according t claim 19. 21. A kit for the diagnostic of Shigella flexneri type 2a infection wherei said kit comprises a molecule according to claim 19. 15 22. A molecule selected from the group consisting of: $\{B(E)C\}$ -O-R-Z $\{(E)CD\}$ -O-R-Z ${AB(E)C}-O-R-Z$ 20 $\{B(E)CD\}$ -O-R-Z $\{(E)CDA\}-O-R-Z$ ${DAB(E)C}n-O-R-Z$ $\{B(E)CDA\}$ n-O-R-Z {(E)CDAB}n-O-R-Z 25 $\{AB(E)CD\}$ n-O-R-Z {DAB(E)CD}-O-R-Z $\{B(E)CDAB(E)C\}-O-R-Z$ wherein: A is an alphaLRhap-(1,2) residue 30 B is an alphaLRhap-(1,3) residue C is an alphaLRhap-(1,3) residue E is an [alphaDGlcp-(1,4)] residue D is a betaDGlcNAcp-(1,2) residue R is an alkyl group comprising 1 to 12 carbon atoms, preferably 1 to 35 carbon atoms, and wherein n is an integer comprised between 1 and 10 And Z is a functional group. 23. A molecule according to claim 22, selected from the group consistin

20

25

30

 $\{B(E)C\}$ -O-R-NH₂ $\{(E)CD\}$ -O-R-NH₂ $\{AB(E)C\}$ -O-R-NH₂ $\{B(E)CD\}$ -O-R-NH₂ 5 $\{(E)CDA\}-O-R-NH_2$ $\{DAB(E)C\}$ n-O-R-NH₂ $\{B(E)CDA\}$ n-O-R-NH₂ {(E)CDAB}n-O-R-NH₂ {AB(E)CD}n-O-R-NH₂ 10 {DAB(E)CD}-O-R-NH₂ $\{B(E)CDAB(E)C\}$ -O-R-NH₂ wherein: A is an alphaLRhap-(1,2) residue B is an alphaLRhap-(1,3) residue 15 C is an alphaLRhap-(1,3) residue E is an [alphaDGlcp-(1,4)] residue D is a betaDGlcNAcp-(1,2) residue

R is an alkyl group comprising 1 to 12 carbon atoms, preferably 1 to 6 carbon atoms, and wherein n is an integer comprised between 1 and 10.

- 24. A monoclonal IgG antibody immunoreactive with a serotype 2a-specific antigenic determinant of the O-SP of *S. flexneri* type 2a, which is produced by an hybridoma cell line deposited under the accession number I-3197, I-3198, I-3199, I-3200 or I-3201, on april, 20, 2004, at the Collection Nationale de Cultures de Microorganismes, INSTITUT PASTEUR, 25 rue du Docteur Roux, 75724 PARIS CEDEX 15, FRANCE.
- 25. A chimeric antibody comprising: (i) a fragment of the heavy and/or light chain(s) which is identical with or homologous to the sequences of one of the mouse monoclonal IgG antibodies according to claim 24, and (ii) the remainder of the heavy and or light chain(s) which is identical with or homologous to the sequences of an antibody from another species or belonging to another antibody class or subclass.
- 26. The chimeric antibody of claim 25, which is a humanized antibody wherein the residues from one or more CDR(s) are replaced by residues from one or more CDR(s) of one of the mouse monoclonal IgG antibodies according to claim 24.
 - 27. The chimeric antibody of claim 26, which is an IgG or an IgA.
- 28. The chimeric antibody of claim 25, which comprises a Fab fragment from said mouse monoclonal IgG antibody and a constant region from a human IgA, or at least the CH3 domains thereof.

15

30

35

- 29. A fragment of the monoclonal antibody according to claim 24, which is a Complementarity-Determining-Region defined by the sequences SEQ ID NO: 12 to 34.
- 30. A polynucleotide encoding the heavy and/or light chain of an antibody according to anyone of claims 24 to 28, or a fragment thereof according to claim 28.
 - 31. An expression vector comprising the polynucleotide according to claim 30.
- 32. An host cell which is modified by a polynucleotide according to claim 28 or a vector according to claim 31.
 - 33. A non-human transgenic animal or a transgenic plant, wherein all or part of the cells are modified by a polynucleotide according to claim 30 or a vector according to claim 31.
 - 34. A pharmaceutical composition comprising an antibody according to anyone of claims 24 to 28 or a functional fragment thereof, and a physiologically acceptable vehicle.
 - 35. A kit for the diagnostic of *Shigella flexneri* type 2a infection, wherein said kit comprises an antibody according to anyone of claims 24 to 28 or a functional fragment thereof.
- 36. A process of preparation of a conjugate according to claim 1 comprising the direct covalent reaction of a molecule according to claims 22 or 23 with an appropriate carrier.
 - 37. A process of preparation of a conjugate according to claim 36 wherein said covalent reaction comprises the previous activation of the molecule.
- 25 38. A process of preparation of a conjugate according to claim 36 wherein said covalent reaction comprises the previous activation of the carrier.
 - 39. A process of preparation of a conjugate according to claim 1 comprising the following steps:
 - a) the conjugation of the molecule according to claims 22 or 23 to a linker to obtain a linker-derivatized molecule,
 - b) the reaction of said linker-derivatized molecule to the carrier.
 - 40. A process of preparation of a conjugate according to claim 1 comprising the following steps:
 - a) the conjugation of an appropriate carrier to a linker to obtain a linkerderivatized carrier,
 - b) the reaction of said derivatized carrier to a molecule according to claims 22 or 23.

WO 2005/003775 PCT/IB2004/002657

149

- 41. A process of preparation of a conjugate according to claims 39 or 40, wherein the linker is a small molecule having a molecular weight of approximately <500 daltons.
- 42. A process of preparation of a conjugate according to claims 39 to 41, wherein the linker is a homobifunctional molecule.
 - 43. A process of preparation of a conjugate according to claims 39 to 41, wherein the linker is a heterobifonctional molecule.